

Supplementary information

Genome-wide identification and characterization of reference genes with different transcript abundances for *Streptomyces coelicolor*

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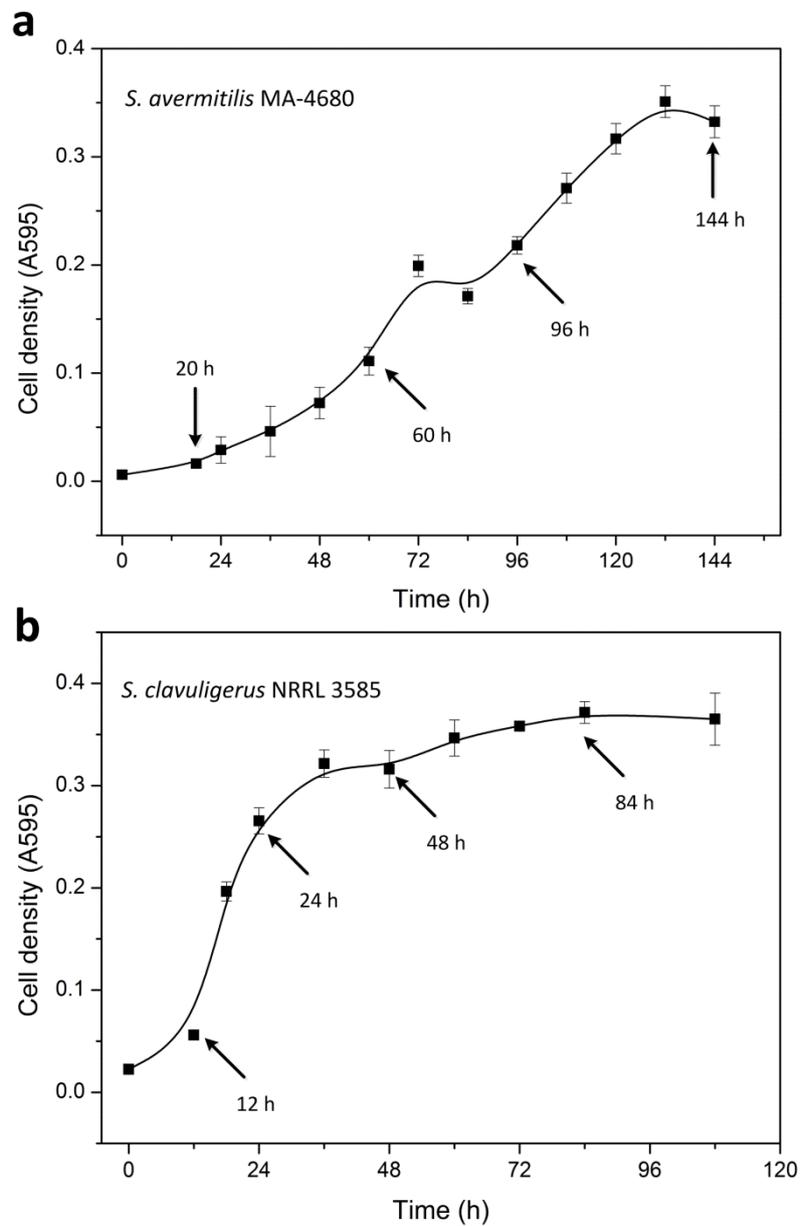


Figure S1 Growth curve of *S. avermitilis* MA-4680 (a) and *S. clavuligerus* NRRL 3585 (b). Cell growth was determined by diphenylamine colorimetric assay at 595 nm¹. Arrows indicate the sampling time points for real-time qRT-PCR.

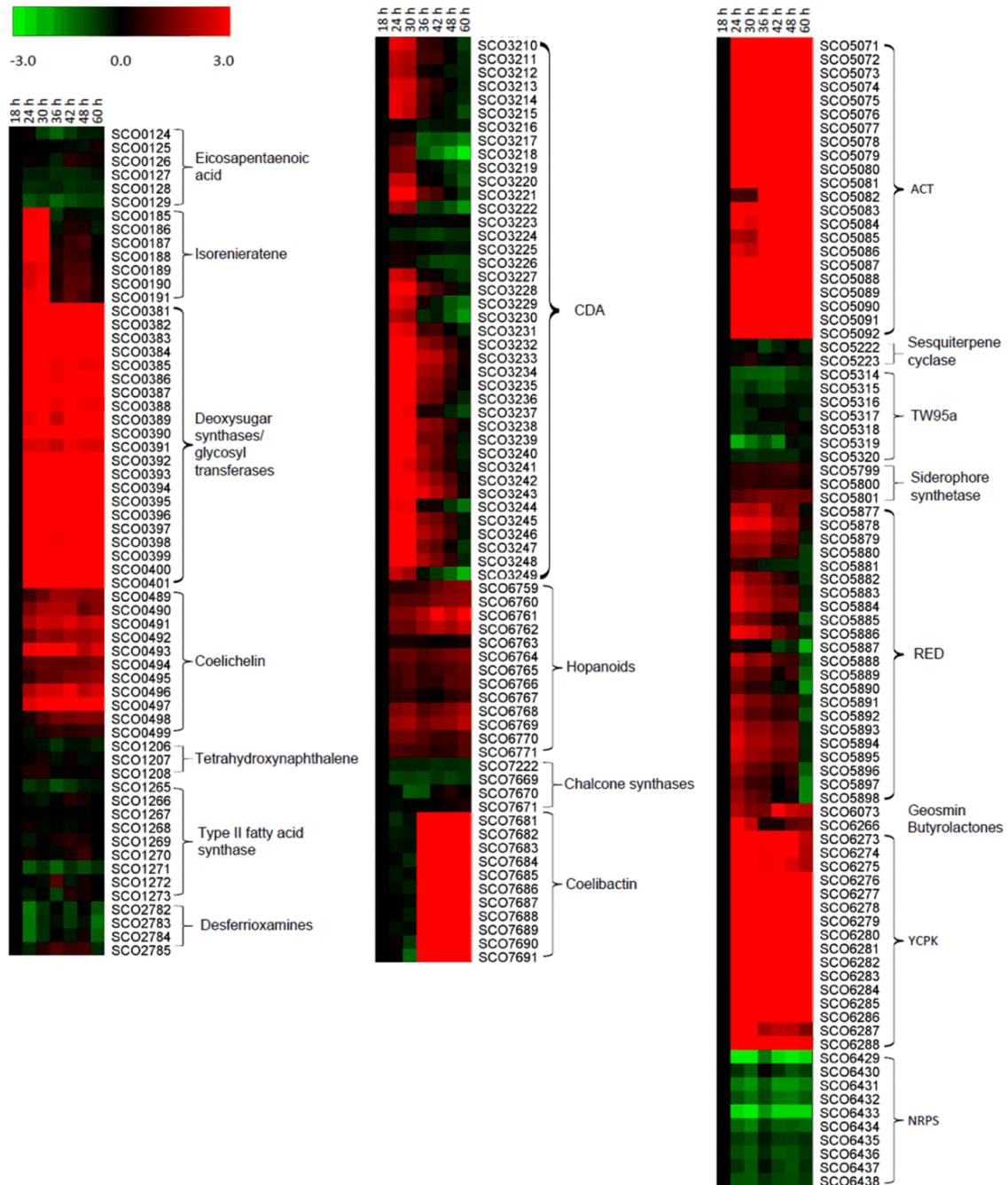


Figure S2 Time-series expression profiles of genes in the biosynthetic clusters of secondary metabolites in *S. coelicolor* M145 cultivated in liquid SMM (GSE53562).

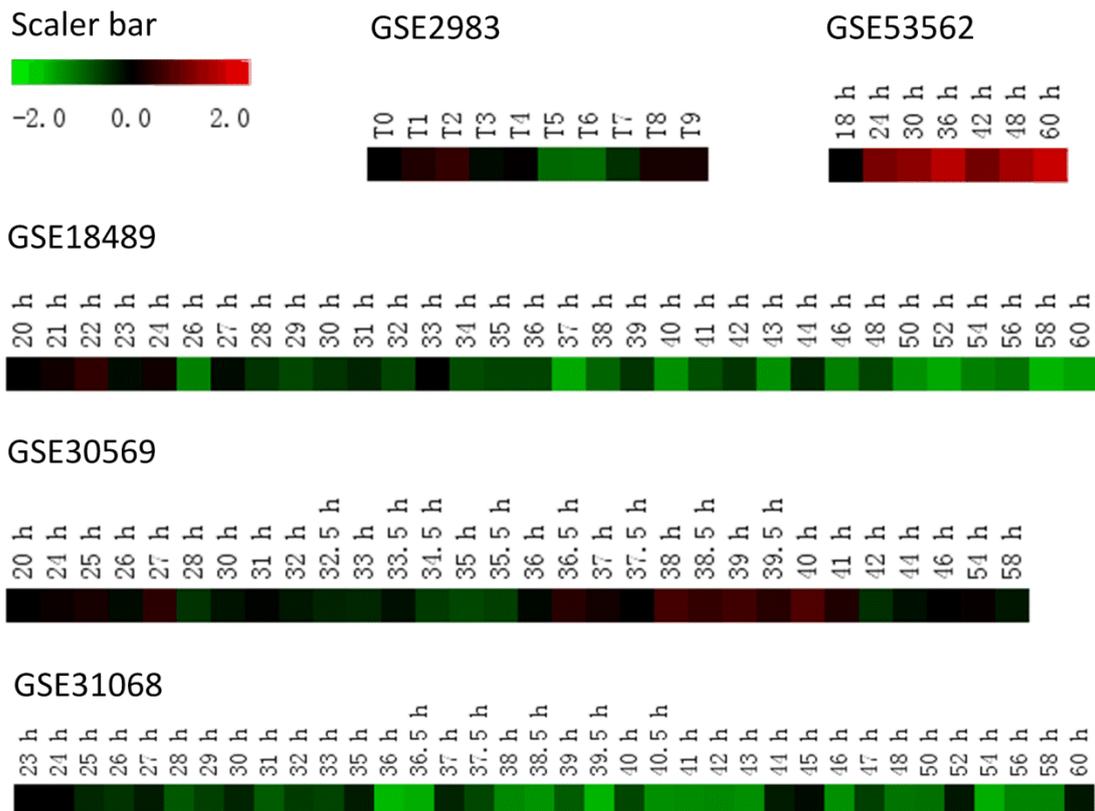


Figure S3 Time-series expression profiles of *hrdB* in four microarrays obtained from growth in different culture conditions. Gene *hrdB* shows unstable expression profiles in dataset GSE18489, GSE31068 and GSE53562. Detailed sampling time of dataset GSE2983 was described by Huang et al.².

Supplementary Tables

Table S1 Overview of all transcriptome microarray datasets used in the present work

Microarray	Strain	Culture medium (liquid)	Number of sampling time points	Number of genes ^a	Source
GSE2983	<i>S. coelicolor</i> M145	Modified R5 medium	10	4,960	Huang et al. ²
GSE18489	<i>S. coelicolor</i> M145	fermentation medium	32	7,893	Nieselt et al. ³
GSE30569	<i>S. coelicolor</i> M145	SSBM-E	30	7,893	Waldvogel et al. ⁴
GSE53562	<i>S. coelicolor</i> M145	SMM	7	7,729	This study
GSE30570	The <i>glnK</i> mutant (SCglnK-3) of <i>S. coelicolor</i> M145	SSBM-E	16	7,893	Waldvogel et al. ⁴
GSE31068	The <i>phoP</i> mutant (INB201) of <i>S. coelicolor</i> M145	The same as GSE18489	36	7,893	Thomas et al. ⁵
GSE53563	<i>S. coelicolor</i> M145 and its <i>scbR2</i> knock-out mutant	SMM +/- jadomycin B	1	7,729	This study

^a The number of genes in the chromosome of *S. coelicolor* M145.

Table S2 Values of coefficient of variance (CV) of the tested candidates in different *Streptomyces* strains

<i>S. coelicolor</i>	M145	M1146	<i>S. avermitilis</i> MA-4680	<i>S. clavuligerus</i> NRRL 3585
Gene ^a	CV	CV	Gene	CV
SCO3183	0.008	0.010	SAV_3674	0.006
SCO6185	0.010	0.013	SAV_4006	0.015
SCO0710	0.012	0.013	SAV_6164	0.021
SCO1544	0.022	0.012	SAV_6806	0.041
SCO4758	0.026	0.021	SAV_4988	0.031
SCO0301	0.017			
SCO2543	0.024			
SCO1962	0.030			
SCO2742	0.032			
SCO6218	0.035			
SCO1453	0.036			
SCO1519	0.041			
SCO1596	0.048			
<i>hrdB</i>	0.052	0.029	SAV_2444	0.051

^a Genes listed in the same row are the orthologs.

Table S3 Orthologs of the five selected RGs in other *Streptomyces* species with complete genome

Strain ^a	Orthologs of the five selected RGs (indicated by locus tag) ^b				
<i>Streptomyces coelicolor</i> A3(2)	SCO0710	SCO1544	SCO6185	SCO3183	SCO4758
<i>Streptomyces avermitilis</i> MA-4680	SAV_6164	SAV_6806	SAV_4006	SAV_3674	SAV_4988
<i>Streptomyces clavuligerus</i> NRRL 3585	SCLAV1201	SCLAV0772	SCLAV3213	SCLAV2320	SCLAV3712
<i>Streptomyces ablus</i> J1074	XNR_4871	XNR_5311	XNR_3380	XNR_2130	XNR_3821
<i>Streptomyces venezuelae</i> ATCC 10712	SVEN_1383	SVEN_1144	SVEN_3952	SVEN_3028	SVEN_4465
<i>Streptomyces</i> sp. PAMC26508	F750_5935	F750_1331	F750_3943	F750_3034	F750_4352
<i>Streptomyces</i> sp. SirexAA-E	SACTE_5642	SACTE_0948	SACTE_6037	SACTE_2644	SACTE_4052
<i>Streptomyces bingchengensis</i> BCW-1	SBI_07973	SBI_02423	SBI_05009	SBI_06485	SBI_04791
<i>Streptomyces cattleya</i> NRRL 8507	SCAT_RS29700	SCAT_RS33190	SCAT_RS32480	SCAT_RS18420	SCAT_RS25550
<i>Streptomyces collinus</i> Tu 365	B446_RS10440	B446_RS07875	B446_RS04785	B446_RS16505	B446_RS22575
<i>Streptomyces davawensis</i> JCM 4913	BN159_RS04320	BN159_RS34915	BN159_1473	BN159_5085	BN159_RS17765
<i>Streptomyces pratensis</i> ATCC 33331	SFLA_RS04490	SFLA_RS26395	SFLA_RS14085	SFLA_RS18365	SFLA_RS12045
<i>Streptomyces fulvissimus</i> DSM 40593	SFUL_1225	SFUL_1054	SFUL_1903	SFUL_2836	SFUL_4589
<i>Streptomyces griseus</i> NBRC 13350	SGR_1186	SGR_5993	SGR_3993	SGR_4295	SGR_2745
<i>Streptomyces hygroscopicus</i> jinggangensis	SHJG_RS17655	SHJG_RS15085	SHJG_RS11535	SHJG_RS23440	SHJG_RS29695
<i>Streptomyces rapamycinicus</i> NRRL 5491	M271_36375	M271_10530	M271_27400	M271_19940	M271_28285
<i>Streptomyces scabiei</i> 87.22	SCAB_RS32925	SCAB_RS35720	SCAB_RS23810	SCAB_53121	SCAB_RS17075
<i>Streptomyces violaceusniger</i> Tu 4113	Strvi_7083	Strvi_3001	Strvi_3450	Strvi_1100	Strvi_8677

^a Strains are those have complete genomes.

^b Make sure the chosen genes have no paralog in one genome before being used as RGs.

Table S4 List of primers used for real-time qRT-PCR in the present work

Primer name	Oligonucleotides (5' to 3')	Product length (bp)
SCO1453-F	GGCAGTACGACATCGGCAAGG	86
SCO1453-R	GCGCTCGAACACCCACAGG	
SCO2543-F	CTCCCGCTCATCGCCTACCA	168
SCO2543-R	GCCGTTGAAGAACAGGAAGTCGT	
SCO1962-F	CGGAGTTCTCCACCGTGCAGC	115
SCO1962-R	GGGTGATCGGCAGGAAGTAGTCG	
SCO4758-F	ATCACCGACCGGATGCCCTT	95
SCO4758-R	GCCGAGCCCCGCTTCTTC	
SCO1596-F	AAGCGGCTGATGCTGGAGAC	91
SCO1596-R	CGGAGTCGATCCGGGAGAT	
SCO6185-F	TGCACGCCCACTTCTGGATGTC	147
SCO6185-R	CGATGCGCTCGTAGGGGCTG	
SCO1544-F	TCGAGGTCGCCCGGGAAGT	140
SCO1544-R	GATCACGTAGGTGGGGGTGCC	
SCO0710-F	TGTCCGCCCTCCGCTCCGTGTCC	172
SCO0710-R	TCCAGGACCGTGTCGCCGTAG	
SCO0301-F	CGGCACCGAACGGCATCTC	65
SCO0301-R	CTGCGTCAACTCGCTGAACCACA	
SCO3183-F	GGGCACCCTCGCGCTCC	96
SCO3183-R	TACTCGCCCCAGTCCAGGTCG	
SCO2742-F	CCCACCGCAACCTGCTCTTCA	101
SCO2742-R	GACTCCCGCCCCTTCAGCC	
SCO6218-F	CGTTCGTACGCCCTTCC	108
SCO6218-R	CCTCGTAGCCGCGTAGTC	
SCO1519-F	GTCCGCGACTACGCCAGGTCAAGG	203
SCO1519-R	ACTGTCTCACGCTCCTCCCCACGG	
<i>hrdB</i> -F	CCTCCGCCTGGTGGTCTCG	132
<i>hrdB</i> -R	AACTTGTAGCCCTTGGTGTAGTCGAAC	
SAV_6806-F	CGAGGAGCTGGACCGTAAGGGAGA	176
SAV_6806-R	CGATGACATAGGTGGGGGTGCC	
SAV_6164-F	GAGCGCATCGCCGAGGTGA	108
SAV_6164-R	GACGTACGCGTGGTCGGACAGT	
SAV_4988-F	CCCCGAGGAGAAGCGGAAGAT	182
SAV_4988-R	CGTTGTGGTGGGACTTGATGTTG	
SAV_3674-F	CCTTGTGGTGGAGAAGCCGTTG	218
SAV_3674-R	CGAGATCGTTGTCCGCCAGG	
SAV_4006-F	CCCTCATCCCGCTCTTCGC	182
SAV_4006-R	GGCGGCGAGTTTCTGAAGTCCT	
SAV_2444-F	CGGTCAAGGACTACCTCAAGCAGAT	168
SAV_2444-R	TCCTCGGCGATGATCTCCAGC	

SCLAV1201-F	CCGTGCGATACCCGCCTTGT	173
SCLAV1201-R	CGGGGCGGATCGTCTTCAAC	
SCLAV0772-F	CTGTCCTGCCCGACTGCC	127
SCLAV0772-R	CCGACACATAGGAGTGCTTGTTC	
SCLAV2320-F	TCGCCGCGTATGTCTCCGTCG	127
SCLAV2320-R	TACTCCCCCAGTCCAGGTCGTTGT	
SCLAV3213-F	TCCACTCGCACTACTGGCTGTCG	109
SCLAV3213-R	GCGGCGTTCTTGACCTTGGC	
SCLAV3712-F	GACCCGGAGGAGAAGCGGAA	192
SCLAV3712-R	ATTGTGGTGGGACTTGATGTTGGC	
SCLAV4698-F	AGGCCCGCACCATCCGTATC	135
SCLAV4698-R	GGGTCATGTCGAGTTCCTTGGC	

References

- 1 Zhao, Y., Xiang, S., Dai, X. & Yang, K. A simplified diphenylamine colorimetric method for growth quantification. *Appl. Microbiol. Biotechnol.* **97**, 5069-5077, (2013).
- 2 Huang, J., Lih, C. J., Pan, K. H. & Cohen, S. N. Global analysis of growth phase responsive gene expression and regulation of antibiotic biosynthetic pathways in *Streptomyces coelicolor* using DNA microarrays. *Genes Dev.* **15**, 3183-3192, (2001).
- 3 Nieselt, K. *et al.* The dynamic architecture of the metabolic switch in *Streptomyces coelicolor*. *BMC Genomics* **11**, 10, (2010).
- 4 Waldvogel, E. *et al.* The PII protein GlnK is a pleiotropic regulator for morphological differentiation and secondary metabolism in *Streptomyces coelicolor*. *Appl. Microbiol. Biotechnol.* **92**, 1219-1236, (2011).
- 5 Thomas, L. *et al.* Metabolic switches and adaptations deduced from the proteomes of *Streptomyces coelicolor* wild type and *phoP* mutant grown in batch culture. *Mol. Cell Proteomics* **11**, (2012).